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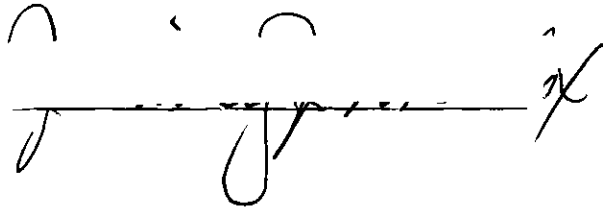

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MITOTIC AND CHROMOSOMAL VARIATIONS IN A CELL CULTURE OF *Potorous tridactylus*

A THESIS

Presented to

The Faculty of the Graduate Division

by

Jimmy Joe Maddox

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IN A CELL CULTURE OF Potorous tridactylus

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Chairman

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SUMMARY

Mitotic and chromosomal alterations and aberrations were investigated in a cell culture of the female Tasmanian rat-kangaroo, Potorous tridactylus obtained from the American Type Culture Collection Cell Repository in Rockville, Maryland. Frequencies of abnormalities were determined in interphase, anaphase, and metaphase stages of cell division by visual and photomicrographic methods. Ideograms of 97 cells were used to establish mean measurements of the relative length (R.L.), centromere index (C.I.), and arm ratio (A.R.). Morphological agreement between homologues was studied. The frequency of anaphase cells having single and double bridges, single and double fragments was determined. Numbers of micronuclei in interphase cells, multipolarity of anaphase cells, and polypoidy in metaphase cells were investigated. Data of this investigation were compared with measurements reported in the literature on primary leucocytes and on another adapted cell line.

The modal chromosome class of this cell line was found to be 11 with 46 per cent of the cells having this number. It was concluded that the incidence of abnormalities was high in this adapted cell line in all of the mitotic stages investigated. Extensive alterations have occurred within the quasidiploid genome (2N = 11) of this rat-kangaroo cell line. The alterations mostly involve pair number 1 and 2 and the loss of an autosomal chromosome from pair 5. The level of abnormalities suggest that this cell line may be undergoing cytogenetic change. Pairing of the four longest chromosomes in this cell line has been obscured by

these alterations and has rendered the order of pairing strictly arbitrary. It is this author's conclusion that the evolution of this adapted cell line has rendered it unsatisfactory for detailed cytogenetic research due to the high level of abnormalities, the inability to distinguish between chromosome pairs 1 and 2, and the absence of an autosomal chromosome in pair 5.

CHAPTER I

INTRODUCTION

Cells maintained *in vitro* undergo many types of changes (Hsu, 1961). Transformation involving genome number, cell type, chromosome morphology, and heteroploidy are common of such cell populations (Hsu, 1961; Levan and Biesels, 1958). To permit the use of such cell lines in biological investigation, the normal level of abnormalities should be investigated and defined. The purpose of this investigation is to elucidate some of the differential cytogenetic parameters in a cell culture of the female Tasmanian rat-kangaroo, *Potorous tridactylus*, which was obtained from the American Type Culture Collection Cell Repository in Rockville, Maryland.

Cytogenetic studies are greatly simplified when there are few chromosomes and they are morphologically distinct. Marsupials are a promising source of mammalian cell lines which meet these two features (Sharman, 1961). Biggers *et al.*, (1965) have reported the diploid chromosome number for four American species, *Caluromys derbianus* (14), *Marmosa mexicana* (14), *Philander opossum* (22), and the common opossum, *Didelphis marsupialis* (22). The rat-kangaroo, *Potorous tridactylus*, used in these investigations has a complement of five pairs of autosomes with XY_1Y_2 sex chromosomes in the male and XX in the female (Sharman, 1950; Sharman and Barber, 1952). The X chromosome is readily identifiable by poorly staining heterochromatic regions proximal to the

centromere in the long arm. Autoradiographic studies of D.N.A. synthesis in the sex chromosomes of the rat-kangaroo, show that the portion of the long arm distal to the heterochromatic regions is synchronous with the autosomes (Hayman and Martin, 1965). This confirms a theory of autosomal translocation to the X chromosome which was first put forth by Sharman, McIntosh, and Barber (1950) as a result of meiotic studies of spermatogonial tissue. It was noted that the short arm portion of the X chromosome paired with the Y_1 and the distal portion of the X long arm paired with the Y_2. A hypothesis was put forth of an ancestral XY system and noted that possibly this animal was undergoing evolution on a chromosomal level at a rapid rate.

It has been shown that extensive chromatid exchanges do occur in the autosomes of Potorous tridactylus by labeling with tritiated thymidine (Walen, 1965). Though cell cultures in general exhibit transformation, mosacism has been found in bone marrow of the marsupial, Schoinobates volans (Hayman and Martin, 1965). This particular cell line of a female specimen has lost one of its small autosomal metacentrics in the fifth pair (Walen and Brown, 1962; Levan et al., 1966).

Primary cell cultures of Potorous tridactylus have been used to establish its exact karyotype (Moore, 1965; Shaw and Krooth, 1964). Due to the transforming characteristics of in vitro culture and the relatively few numbers of transfers, it is desirable to establish the karyotype parameters of this particular cell line and compare it with the reported typical diploid karyotype.

Detailed analysis of the karyotype from examination of metaphase cells was performed. The Denver System of nomenclature will be used to

define the karyotype of this adapted cell culture (Robinson, 1961). All pairs are arranged in a series of decreasing length with the sex chromosomes segregated from the autosomes.

The relative length (R.L.) of each chromosome has been determined as the length of the particular chromosome divided by the total length of an X-containing haploid set multiplied by 100 to express it as per cent length.

The centromere index will be calculated to establish the location of the centromere. The centromere index (C.I.) is defined as the length of the short arm divided by the total length of the chromosome multiplied by 100.

A third parameter is the arm ratio (A.R.). This is defined as the length of the long arm of the chromosome divided by the length of the short arm. It is understood that the arm ratio and centromere index are related algebraically but it is desirable to establish both for the sake of clarity.

Adapted cell lines often show morphological differences between members of a pair, therefore, it has been established how well the members of a pair agree with each other. This was established by dividing the length of one member into the other and determining if the resulting figure differs significantly from one.

Through detailed analysis of ideograms, the near diploid formulae was investigated. This has shown which chromosomes are involved in aneuploid and pseudodiploid formulae. From such an analysis varying degrees of responsibility can be assigned to the chromosomes which produce the abnormalities of the cell line.

Cells in mitotic anaphase division have been studied to show the frequency of fragments and bridges present in the adapted cell line. The frequency of single fragments corresponds to the frequency of chromatid breakage (Lea, 1955). Single bridges and double bridges correspond to the frequency of dicentric chromosomes, and therefore give rise to chromosomes which may result from a breakage-fusion-breakage cycle (McClintock, 1929). Anaphase analysis will also show the frequency of multipolar divisions. Fragments which persist into interphase often show up as micronuclei of a cell and the incidence of micronucleated cells shares some correlation with fragmentation of the chromosomes.

CHAPTER II

MATERIALS AND METHODS

Source of Material

Kidney cells of a female rat-kangaroo (Potorous tridactylus, CCL-35) were obtained from American Type Culture Collection Cell Repository in Rockville, Maryland (Walen and Brown, 1962). The cells were grown in a medium composed of Eagles' amino acid and vitamin solution with non-essential amino acids and sodium pyruvate added (Eagle, 1959) in Hank's salt solution and 20 per cent fetal calf serum with penicillin and streptomycin at 50 milligrams per liter. Cultures were incubated at 35°C. Stock cell supplies were maintained in 5 ml sealed glass ampules containing 60 per cent the above media, 30 per cent fetal calf serum, and 10 per cent glycerol and kept frozen in liquid nitrogen canisters.

Slide Preparation

The cells were thawed from stock supplies as needed and grown in wide mouth French square bottles into which a microscope slide had been placed. Cells were transferred by scraping the culture bottle with a silicone policeman. Sterile techniques were applied at every stage of the transfer procedures.

A uniform cell sheath was permitted to grow over the microscope slide in the course of four or five days. All slides were examined for this uniform cell sheath by using an inverted microscope. Metaphase

cells were arrested by introducing sufficient colchicine from a stock solution to give a final concentration of 2 milligrams per liter. Cells were then hypotonically treated in 20 per cent growth medium and 80 per cent distilled water, kept at a constant temperature (35°C) for 20 minutes, and then fixed by immersion into a Coplin jar filled with a fixative solution of 3 parts ethanol and 1 part acetic acid for several hours. The Coplin jar solution with the preparation was chilled in a freezer of dry-ice for five minutes. Excess fixative was drained from the slide and that remaining was flamed from the slide. This flaming process makes the cells adhere to the slide while the cooling prevents excessive heat transfer to the cells.

The slide was stained with natural orcein in 50 per cent propionic acid. This stain was freshly prepared each day by boiling a 50 per cent propionic acid solution saturated with natural orcein and filtering after the solution had cooled. After staining, a coverslip was applied and excess stain blotted away. The coverslip was then sealed by applying printer's wax.

Slides made in this fashion are temporary slides; they are suitable for analysis for a period of about six to eight weeks. Anaphase preparations were made the same way omitting the colchicine and hypotonic treatments. This method is modified from Ford and Hamerton (1956).

Analysis

All microscope studies were made with an Officine Galileo phase contrast microscope at a magnification of 1500x. Photomicrographs were

made with an Exakta 35mm camera using Kodak Panatomic-x film. For the determination of chromosome numbers, slides were scanned and only those cells which were flat and had minimal chromosome overlap were counted. For detailed analysis, pictures were taken of apparently diploid cells without counting the chromosomes. The film was developed with Kodak microdal developer and projected on graph paper by means of a lamp projector. The paper was oriented in the proper fashion and the chromosomes were traced onto the paper using a rapidograph pen with a size 00 point. Measurements were made with a ruler calibrated to 1/50 of an inch. No effort was made to magnify all figures to the same extent since the variation of absolute chromosome lengths among different cells allows only relative measurements of chromosomal length.

CHAPTER III

RESULTS

The modal distribution of the chromosome number per cell can be seen in Table 11 (Appendix) and Figure 1. The modal chromosome number is 11 with 46.9 per cent of 210 cells having this complement. Approximately 83 per cent of the cells analyzed had chromosomes in the 9 - 14 range. The theoretical triploid number of a complement of 11 chromosomes could be either 16 or 17. Both complements of 16 and 17 had a frequency of 0.5 per cent each and the 18 chromosome complement had a frequency of 2.4 per cent. The apparent tetraploid cells (8.1 per cent) were found to have 20 - 24 chromosomes. Four point five per cent of the cells exhibited higher chromosome numbers; this included one octaploid complement.

Results of 502 anaphase analyses can be seen in Table 1. A cell undergoing dipolar division without fragments of deleted chromosomes or chromatids and without bridges was considered a normal anaphase figure. These criteria for a normal anaphase division were met by only 70.52 per cent of the 502 cells analyzed. The frequency of deletions and bridges of the abnormal anaphase cells do not add up to the total frequency of abnormal anaphase cells, 26.25 per cent, since some cells had both a deletion and a bridge. The values for deletions and bridges include only dipolar cells since the frequency of both abnormalities is much higher in multipolar cells. The single bridges (10.63 per cent) had a frequency of twice that of double bridges (5.63 per cent) while both single and

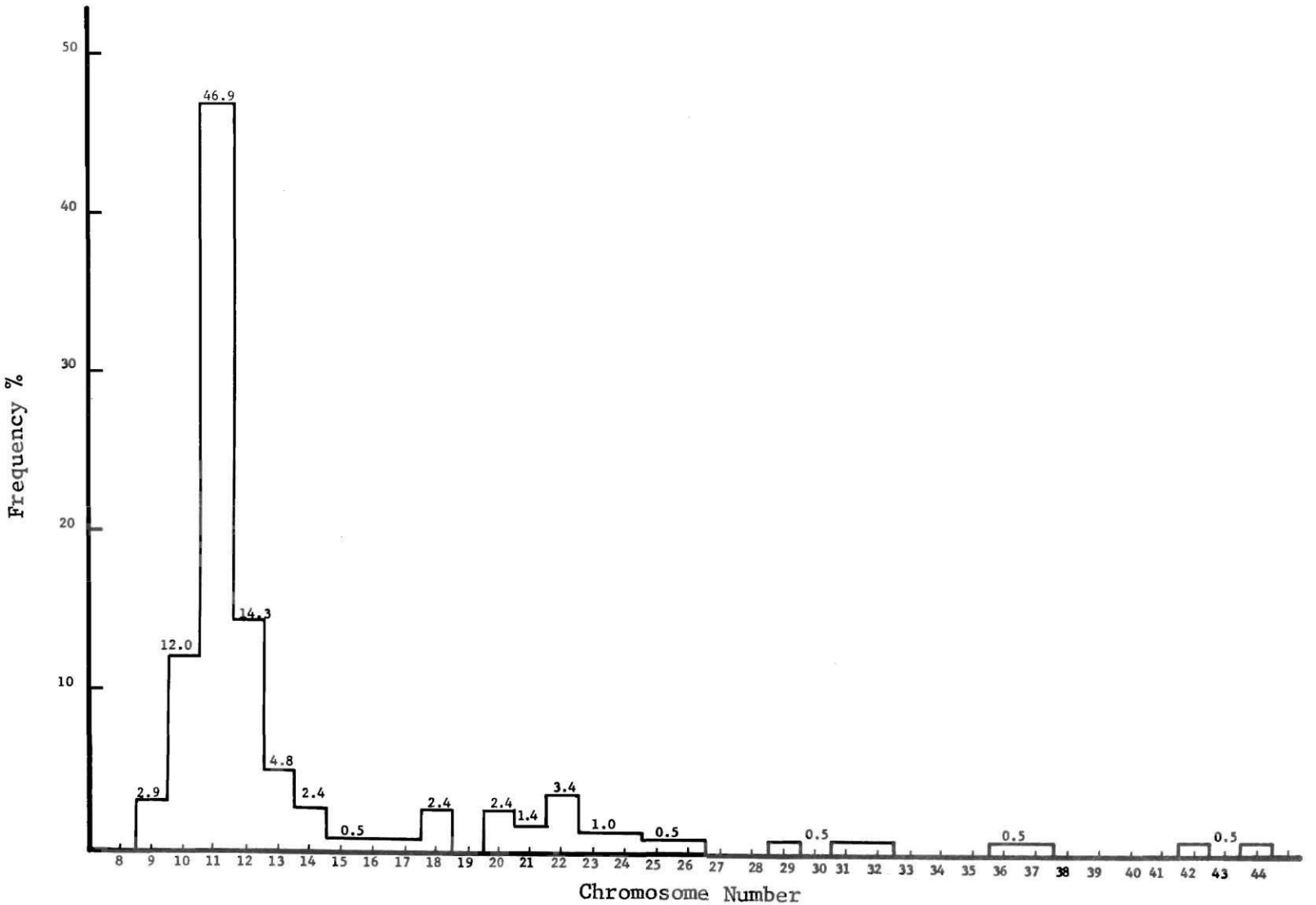


Figure 1. Frequency Histogram of Chromosome Number Per Cell of 210 Cells.

double deletions were nearly equal to the frequency of double bridges. The frequency of multipolar division was 4.38 per cent.

Table 1. Anaphase Analysis of 502 Cells

	Normal[1]	Abnormal Dipolar	Multipolar	Deletions[2]		Bridges[2]	
				Single	Double	Single	Double
Number	354	126	22	30	35	51	27
Per cent	70.52	26.25	4.38	6.25	7.29	10.63	5.63

[1]Refer to text for criteria of a normal cell.

[2]Includes only dipolar cells.

Observed numbers of micronuclei of 981 interphase cells are tabulated in Table 2. Cells with only 1, 2, and 3 micronuclei were observed. The total frequency of the micronuclei was 5.4 per cent. This is approximately one-half the frequency of both single and double deletions recorded in Table 1. It appears that approximately one-half of the fragments whether chromatid or chromosome fragments persisted through cell division to appear as micronuclei in the interphase cells.

Table 2. Frequency of Micronuclei in Interphase Cells

	Total	Number of Micronuclei			
		0	1	2	3
Number of cells	981	926	41	11	3
Per cent	100.0	94.6	4.2	1.1	0.1

The karyotype of this Potorous tridactylus cell line (CCL-35) is

shown in Figure 2a and 2b. The number 1 and number 2 pairs offer no consistent means for pairing because of the large morphological differences seen in the four longest chromosomes. These four chromosomes were labeled 1a, 1b, 2a and 2b in descending order of their relative lengths. Pairing of these four chromosomes according to matching relative lengths and long arm lengths is shown in Figure 2a. If the short arm lengths and centromere indices, are considered as pairing criteria, chromosome 1a - 2a and 1b - 2b would have to be paired as homologues as shown in Figure 2b. The pairing of these four chromosomes, at present, is strictly arbitrary.

Table 3 shows mean measurements of 77 cells with a diploid complement of 11 chromosomes. The values of the three criteria for identification are shown, relative length, centromere index, and arm ratios with their respective standard errors. Standard errors were calculated according to the method of Croxton (1959). The average values of the number 1 and number 2 pairs are also shown along with the individual values of pairs 1 and 2. The short arms of 1b and 2b are nearly equal, however, their centromere indices are different due to the large difference in the length of their long arms.

There is considerable overlap among the measurements on different chromosomes (Table 4, Figure 3). Because of this overlap it was necessary to test for significance of the differences among chromosomes and pairs of chromosomes. Figure 3 is a plot of relative length versus centromere index according to the method of Shaw and Krooth (1964). The absolute ranges of each shown by the rectangles. One can see that the measurement ranges do not allow quantitative distinction between some chromosomes, especially those of 3, X, and 2b. A similar plot is shown

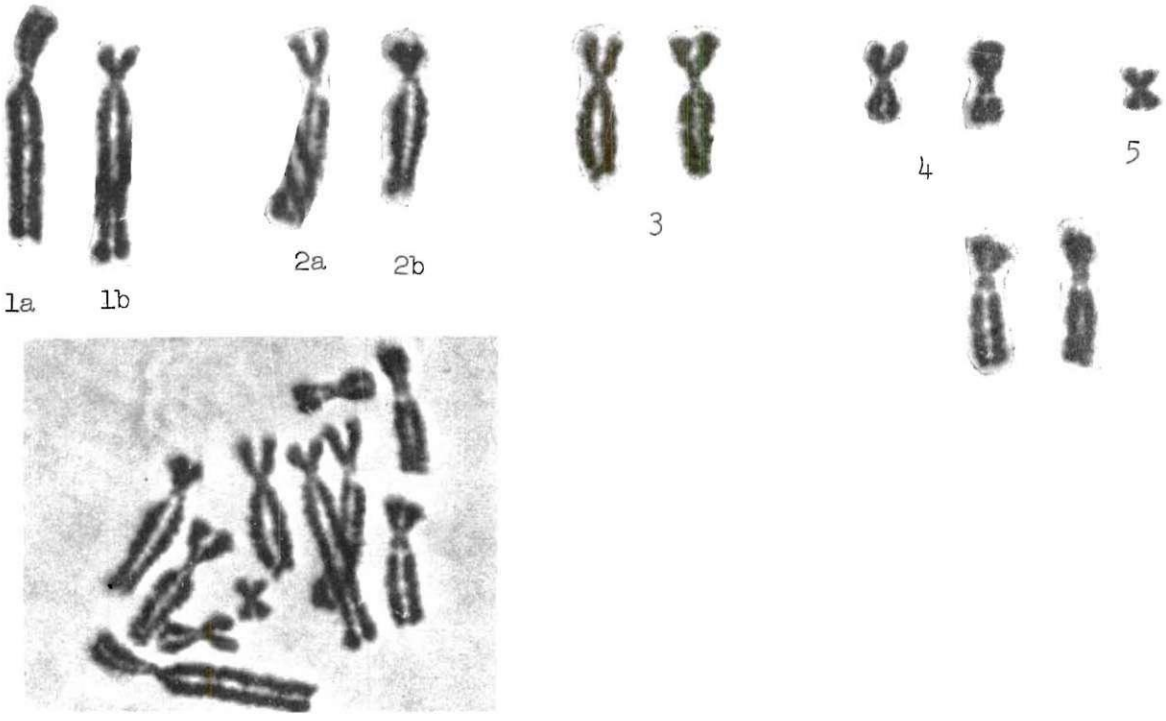


Figure 2a. Karyotype of Potorous tridactylus Adapted Cell Line CCL-35 Showing Pairing of 1a - 1b and 2a - 2b.

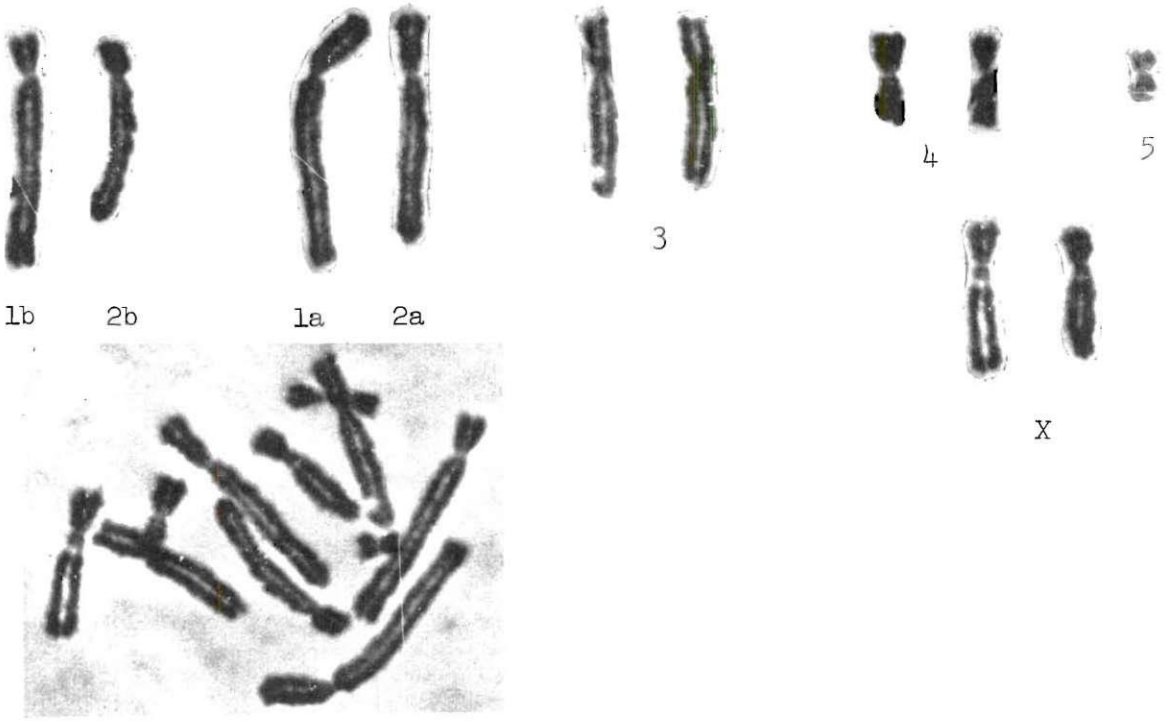


Figure 2b. Karyotype of Potorous tridactylus Adapted Cell Line CCL-35 Showing Pairing of 1b - 2b and 1a - 2a.

in Figure 4 showing 99.9 per cent confidence intervals around the means. This statistical method distinguishes clearly among the eight chromosome groups.

Table 3. Mean Chromosome Measurements of 77 Cells

Chromosome	Relative Length[1]	Centromere Index[2]	Arm Ratio[3]
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
1a	30.95 ± 0.23	26.65 ± 0.49	2.89 ± 0.09
1b	26.95 ± 0.21	17.83 ± 0.49	4.97 ± 0.19
	$\bar{X}a,b$ = 28.95 ± 0.10	$\bar{X}a,b$ = 22.24 ± 0.49	$\bar{X}a,b$ = 3.93 ± 0.14
2a	24.41 ± 0.27	26.20 ± 0.64	2.91 ± 0.08
2b	21.14 ± 0.23	24.60 ± 0.62	3.30 ± 0.15
	$\bar{X}a,b$ = 22.77 ± 0.17	$\bar{X}a,b$ = 25.40 ± 0.63	$\bar{X}a,b$ = 3.11 ± 0.11
3	19.23 ± 0.17	31.80 ± 0.52	2.21 ± 0.05
4	10.05 ± 0.10	46.20 ± 0.19	1.18 ± 0.01
5	5.61 ± 0.03	47.20 ± 0.26	1.15 ± 0.05
X	16.39 ± 0.26	27.40 ± 0.38	2.74 ± 0.06

[1]Per cent of total length of an X containing haploid complement.

[2]Length of short arm x 100/total length of the chromosome.

[3]Length of long arm/length of short arm.

Homologues of pair 3, 4 and X were not readily distinguishable. It was desired to know how one homologue compared to the other. Table 5 shows the agreement of the pair members as determined by dividing the largest homologue of a pair into the shortest homologue to obtain a number less than 1. The closer the number is to 1, the higher the agreement of the chromosome lengths of homologous chromosomes. The pairing of Figure 2a was used to obtain the values in Table 5.

◇, Chromosome 1a; △, Chromosome 1b; □, Chromosome 2a;
⬡, Chromosome 2b; ○, Chromosome 3; ▲, Chromosome 4;
■, Chromosome 5; ●, X Chromosome.

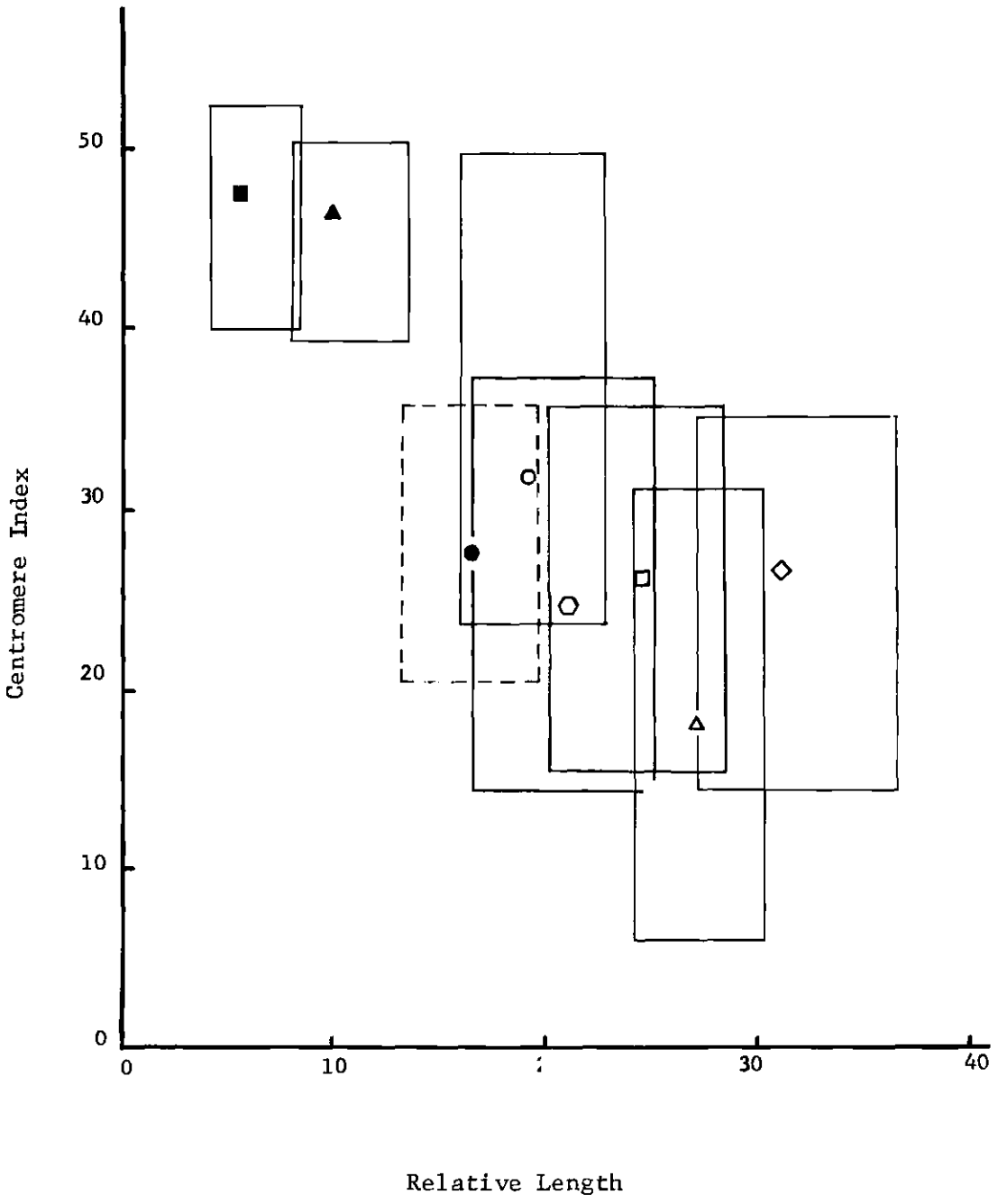


Figure 3. Plot of Relative Length Versus Centromere Index with Rectangles Showing Ranges of Measurements.

◇, Chromosome 1a; △, Chromosome 1b; □, Chromosome 2a;
⬡, Chromosome 2b; ○, Chromosome 3; ▲, Chromosome 4;
■, Chromosome 5; ●, X Chromosome.

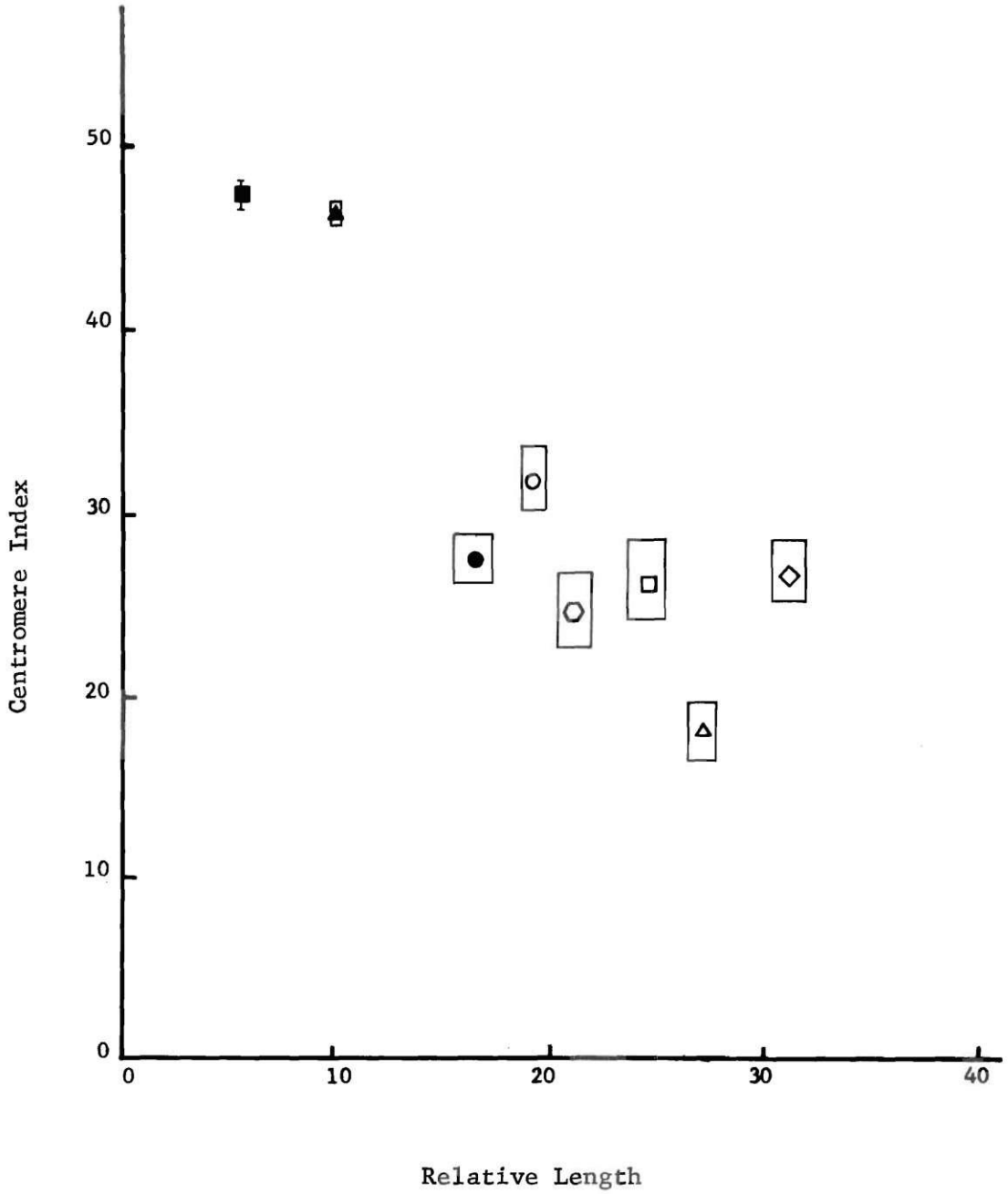


Figure 4. Plot of Relative Length Versus Centromere Index with Rectangles Representing a 99.9 Per Cent Confidence Interval of the Standard Error.

Table 4. Ranges of Mean Measurements of 77 Cells

Chromosome	Relative Length	Centromere Index	Arm Ratio
1a	36.2 - 26.8	35.1 - 14.4	5.96 - 2.03
1b	30.2 - 24.0	31.2 - 6.0	8.77 - 2.16
2a	28.3 - 20.1	35.7 - 15.4	5.06 - 1.00
2b	25.0 - 16.3	37.2 - 14.3	13.00 - 1.43
3	22.6 - 15.8	49.7 - 23.7	3.45 - 1.30
4	13.5 - 8.0	50.3 - 39.4	1.54 - 1.00
5	8.5 - 4.2	52.4 - 40.0	1.50 - 1.00
X	19.7 - 13.2	35.9 - 20.5	5.99 - 1.52

Table 5. Agreement of Homologous Chromosomes[1]

Chromosome Pair	Mean ± S.E.	Coefficient of Variance
1	0.872 ± 0.008	8.14%
2	0.841 ± 0.011	11.29%
3	0.930 ± 0.010	9.03%
4	0.905 ± 0.009	9.17%
5	---	---
X	0.932 ± 0.013	12.87%

[1]Shorter homologue length/longer homologue length for 77 pairs, paired as in Figure 2a.

Ninety-seven apparent pseudodiploid cells in the diploid range were analyzed for their exact chromosome complement. The results of this analysis are seen in Table 6. Cells were superficially analyzed under the microscope for the diploid range (9 - 14) and photographed. The

constructed ideograms were then analyzed to determine what types of rearrangements, deletions, additions, or marker chromosomes were present. Table 6 shows the results of this analysis. Only chromosome complements of 10, 11, 12 and 14 were found. Pseudodiploid formulae were scored when a cell was found to have 11 chromosomes but normal chromosomes were not present. A dicentric chromosome was counted as two chromosomes.

Many arm and chromatid length variations were seen throughout the ideograms. It was decided that a chromosome had suffered a deletion or an addition to either an arm or one chromatid when its relative length varied from the mean measurement by as much as 20 per cent. These data are shown in Table 7. Table 8 represents a summary of all the data on supposed deletions and additions. It was found that chromosomes of pair 4 and 5 were frequently missing entirely while chromosomes of pair 1 and 2 showed most of the deletions and additions. The chromosome with the highest number of abnormalities was chromosome 1a and pair 4. Chromosome 1a was often involved in whole arm deletions of the long arm and additions to the short arm. Abnormalities of pair 4 often involved a whole chromosomal deletion from the pair.

Table 6. Chromosome Formulae of 98 Apparently Diploid Cells

Ploidy	Chromosome Number	Number of Cells	Formula
Hypodiploid	10	1	2N - 2a
		5	2N - 4
		1	2N - 5
		1	2N - 3
Diploid	11	77	2N
Pseudodiploid		1	2N - 3 - 1a + dicentric
		1	2N - 3 - X + small telocentric + large telocentric
		1	2N - 4 - 1b - 2a + dicentric + small telocentric
		1	2N + 4
		1	2N - 4 + small telocentric
		1	2N - X + ring
		1	2N - X - 1b + dicentric
Hyperdiploid	12	1	2N - 2a + X + Marker
		1	2N + 5
		1	2N - X + dicentric
		1	2N + small telocentric
		1	2N + large subtelocentric
	14	1	2N + 1a + 3 + X

Table 7. Apparent Chromosome Deletions and Additions of 98 Diploid and Apparently Diploid Cells[1]

	Deletions				Additions				
	Short Arm Chromatid Arm		Long Arm Chromatid Arm		Short Arm Chromatid Arm		Long Arm Chromatid Arm		Total
1a		1	2	4		4		3	14
1b			1	3	1			1	6
2a	1		2	4			1		8
2b	1	2		3	2	1	1		10
3		2	1	3		1			7
4[2]	2	3			1	1			7
5	-	-	-	-	-	-	-	-	0
X	1	1	1	1	1				5

[1]Twenty per cent deviation from the mean measurements was used as a significance.

[2]Number 4 is metacentric, therefore, no distinction was made between long and short arm.

Table 8. Frequency of Chromosome Abnormalities in Specific Chromosomes

Chromosome	1a	1b	2a	2b	3	4	5	X
Abnormalities	15	8	11	10	10	15	2	10
Per cent	18.5	9.9	13.6	12.3	12.3	18.5	2.6	12.3

CHAPTER IV

DISCUSSION

Cell line CCL-35 of Potorous tridactylus has undergone extensive alterations when compared to the primary cell cultures of the same species analysis of Shaw and Krooth (1964). Measurements of relative lengths and arm ratios are compared in Table 9. Levan et al. (1966) obtained their

Table 9. Mean Measurements of the Karyotype of Potorous tridactylus

Primary Cells (Shaw and Krooth)			Cell Line (Levan, et al.)			Cell Line (These Data)		
Chromosome Number	R.L.	A.R.	Chromosome Number	R.L.	A.R.	Chromosome Number	R.L.	A.R.
1	24.4	4.0	1	29.5	2.4	1a	30.9	2.9
			2	25.4	4.2	1b	26.9	4.9
2	22.7	1.7	3	22.8	2.7	2a	24.4	2.9
			4	19.5	3.0	2b	21.1	3.3
3	18.3	1.9	5	18.6	2.0	3	19.2	2.2
			6	18.1	2.3			
X	17.3	2.1	7	16.1	2.3	X	16.4	2.7
			8	15.9	2.3			
4	10.4	1.1	9	11.1	1.1	4	10.1	1.2
			10	10.4	1.1			
5	6.7	1.1	11	6.4	1.1	5	5.6	1.1

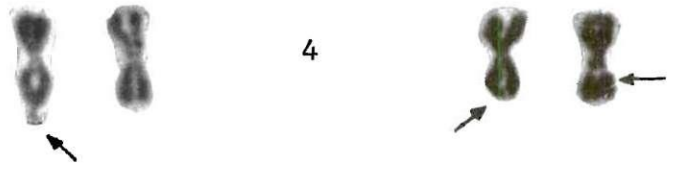
cell line from the Cell Bank of the South Jersey Medical Research Foundation at Camden, New Jersey, while the cell line for this research was obtained from the American Type Culture Collection Cell Repository at Rockville, Maryland. In general, the mean chromosome measurements of this investigation agree with those of Levan et al. (1966). Relative changes from the reported values of Shaw and Krooth (1964) are very similar for

Levan's data and for this investigation. However, the values of this investigation are, in general higher than the mean measurements of Levan's. The generally higher values found in our material as compared to the data of Levan et al. could be accounted for by differences in the methods. Levan gives no standard errors for his data. Therefore, only the mean values can be compared and this may involve considerable error. Furthermore, no statistical reliability of homologue measurements were given by Levan for pairs 3, 4 and X (his number 5 to 9). This author chose not to analyze these pairs on a homologue basis because of the difficulty in distinguishing between the members on the basis of relative length.

Factors other than relative length often allow a distinction between homologue members of a pair. One member of pair 3 and 4 often shows a satellite. In the case of chromosome 3 this satellite is on the long arm (chromosome 4 is metacentric). The homologue of number 4 not showing the satellite often shows a poorly staining heterochromatic area on one arm and frequent deletions seem to occur in this region. The X chromosomes seem to show a difference in overall length. The short arm of one X is slightly shorter than the short arm of its homologue, but the clearest difference shows up in the state of contraction of the two chromosomes.

The absolute ranges of the chromosome measurements overlapped considerably (Figure 3, Table 4). Therefore, even with as few chromosomes as are present in this material it is impossible to rely on individual pair measurements for chromosome identity. However, the parameter values are not random within a cell. This fact is brought out by applying

Satellite



Satellite and Heterochromatic Gaps

Small Difference in Short Arm Length and State of Contraction

Figure 5. Comparison of Homologues of Pairs 3, 4 and X

a "Student-t-Test" for the level of confidence in chromosome distinction. The data of Table 5 are based on measurements of 77 cells. Figure 4 is a plot of the relative length versus the centromere index where the rectangles indicate a standard error of the mean that has been multiplied by a t-value at a 99.9 per cent probability level at an estimated 60 degrees of freedom. It can be seen that no overlap is present in the range values. Chromosome number 5 shows the highest confidence interval as would be expected from the smallest chromosome of the complement. Figure 4 also shows how relatively easy the chromosomes of pairs 1 and 2 can be distinguished. The chromosomal changes during the adaptation of this cell line may have been severe, but have not obliterated the differences among the chromosomes.

Chromosome 2b is sometimes difficult to distinguish from pair 3 on the basis of relative length (Table 3). However, they can usually be told apart in good preparations. The short arm (C.I.) of 2b is much shorter than chromosome 3 and a large difference in the arm ratios helps to distinguish the chromosomes. The X chromosomes may be confused with pair 3 (Shaw and Krooth, 1964) when the secondary constrictions of the X chromosome are not visible. This case is rare.

Since the cell line used for this research has lost one member of pair 5 the total X-containing haploid length is certainly different from the one studied by Shaw and Krooth (1964). This difference should be a constant if the length of chromosome number 5 seen in this cell line was the same as the missing chromosome. No correction factor has been introduced to compensate for this loss, since the missing chromosome might not be entirely absent but partially or completely incorporated in

the present karyotype, such translocations and additions might well account for some alterations of the chromosomes studied.

Levan et al. (1966) have stated that chromosome number 1a has gained material in the short arm. The arm ratio of chromosome 1a reflects this difference when compared to the arm ratio of chromosome 1 of Shaw and Krooth. In comparing differences of the arm ratio of chromosome number 1 with pair 1 and 2 of Shaw and Krooth, one can see that this difference is less for the pair 2 comparison according to Levan and nearly equal for the measurements found in this investigation. Therefore, the possibility of addition of chromosomal material to the long arm of one member of pair 2 must be considered.

Chromosome number 1b has increased in both total length and arm ratio while the centromere index is less than that reported for chromosome 1. This would mean lengthening of the long arm or both long arm lengthening and a short arm deletion, but in either case an increase in R.L. for chromosome 1b as compared to chromosome 1 of Shaw and Krooth. An increase in arm ratio, and relative length with an accompanying decrease in centromere index for chromosome 2a would lead to the same conclusions.

Chromosome number 2b also has an increase in the arm ratio accompanied by a decrease in the centromere index, but a shorter relative length when compared to chromosome 2 of Shaw and Krooth. This indicates a deletion from the short arm or both a deletion in the short arm and an increase in the long arm.

The same conditions and conclusions apply to chromosome number 3 as those of chromosome 1b and 2a. Chromosome number 4 has remained

nearly unchanged while the X chromosome and number 5 appear to have a shortened relative length. The arm ratio of chromosome 5 has remained constant indicating a deletion of equal length from both arms. The arm ratio of the X chromosome pair has increased, leading one to believe that a deletion occurred in the short arm.

Extensive chromatid and arm exchanges have been suggested to occur in cell lines of Potorous tridactylus (Walen, 1965). The abnormalities seen in this investigation suggest that this cell line may be undergoing a transformation that has not yet stabilized. This is evidenced by the finding of more than 20 per cent of a diploid sample of 97 cells showing major alterations from the stemline karyotype! Serious doubt is cast as to the use of establishing a norm for the cell line.

The modal chromosome number of 11, occurred in 46.9 per cent of 210 cells sampled. Levan et al. (1966) found a modal number of 11 in 56.0 per cent of 223 cells sampled. When ideograms of cells supposedly containing between 9 and 14 chromosomes were analyzed, no cells with a chromosome count of either 9 or 13 were detected. Careful analyses of ideograms therefore show less of a spread of chromosome number than counts done under the microscope.

This discrepancy is difficult to explain. It has been pointed out by Ford (1964) that differences encountered between simple chromosome "counts" and careful analyses of ideograms necessitated this distinction between "counts" and "numbers" of chromosomes. It is this author's opinion that the "counted" frequencies of chromosome numbers 9, 10, 12, 13, and 14 are subject to significant error due to the high frequencies of chromosome breakages which often result in large fragments being classified as chromosomes.

Multipolar anaphase abnormalities were about 6 to 10 times as frequent as dipolar anaphase abnormalities (Table 16, Appendix). Tripolar cells often have a frequency of aberrations as high as 12 times the anaphase dipolar aberrations (Giles, 1941). Therefore, this level of aberrations in multipolar divisions is not abnormally high for this type of material. The 26.25 per cent level of abnormal dipolar anaphase cells is comparable with aberration frequencies in mouse fibroblast cultures, which have about a 20 per cent aberration level (Puck, 1958). The frequency of micronuclei in interphase cells was about 5.4 per cent (Table 2). The fragments of anaphase divisions often persist into interphase to show up as micronuclei (Lea, 1955). The level of micronuclei compared with the anaphase data indicates that about one-half of the deletions persisted into interphase. This level of micronuclei in interphase is high when compared to the value of 3.56 per cent in most plant material (Sparrow and Sparrow, 1950).

The findings of this report indicate that this cell line is not a favorable material for detailed cytogenetic studies. The mere fact that pairing of pairs 1 and 2 is strictly arbitrary makes detailed cytogenetic research with this material difficult. If a breakage were seen in chromosome 1a but not in 1b, 2a, or 2b after introduction of a mutagenic agent, then the double effect of normal karyotypes would be lost and the probability of random chance would be the more likely event.

The sum total of all the abnormalities of interphase, anaphase, multipolar, and metaphase analyses are not too far from the usual abnormality level of other cell lines (Hsu, 1961), but the outcome of initial attempts of the cell line to stabilize itself has resulted in a

significant karyotype change from reported values of primary cells. Certainly the animal is a promising source for mammalian cells for cytogenetic studies because of its extremely low diploid number of 2N = 13 for the male and 2N = 12 for the female (Sharman, McIntosh, and Barber, 1950). Potorous tridactylus is superseded only by Protemnodon bicolor (black-tailed wallaby), which has a diploid number of 2N = 10 for the female and 2N = 11 for the male (Sharman, 1961).

CHAPTER V

CONCLUSIONS

The conclusions of this investigation based on factual evidence reported in this thesis are as follows:

1. The incidence of abnormalities is high in this adapted cell line of kidney tissue from the Tasmanian rat-kangaroo, Potorous tridactylus.

2. Extensive alterations within the quasidiploid genome of this rat-kangaroo cell line have occurred and the changes mostly involve pairs number 1 and 2 and the loss of an autosomal chromosome from pair 5.

3. Pairing of the four longest chromosomes in this cell line has been obscured by these alterations and has rendered the order of pairing strictly arbitrary.

4. The evolution of this cell line has rendered it unsatisfactory for detailed cytogenetic research due to the inability to distinguish between chromosome pairs 1 and 2 and the absence of an autosomal chromosome in pair 5.

APPENDIX

Table 10. Chromosome Number of 210 Cells

Chromosome Number	Number of Cells	Per Cent of Total
9	6	2.87
10	25	11.96
11	98	46.89
12	30	14.35
13	10	4.78
14	5	2.39
15	1	0.48
17	1	0.48
16	1	0.48
18	5	2.39
20	5	2.39
21	3	1.44
22	7	3.35
23	2	0.96
24	2	0.96
25	1	0.48
26	1	0.48
29	1	0.48
31	1	0.48
32	1	0.48
36	1	0.48
37	1	0.48
42	1	0.48
44	1	0.48

Table 11. List of Symbols and Formulae Used (Croxton, 1959)

Symbols

Σ - Sum

X - Sample

N - Number of Samples

$\bar{X}$ - Arithemetic Mean

σ - Standard Deviation

$\sigma_{\bar{X}}$ - Standard Error of the Mean

R.L. - Relative Length

C.I. - Centromere Index

A.R. - Arm Ratio

T - Total Length of an X-containing Haploid Complement

L_t - Length of a Chromosome

L_s - Length of Short Arm of a Chromosome

L_l - Length of Long Arm of a Chromosome

Formulae

$$\bar{X} = \frac{\Sigma x}{N}$$

$$\sigma = \sqrt{\frac{\Sigma x^2}{N} - \left(\frac{x^2}{N}\right)}$$

$$\sigma_{\bar{X}} = \frac{\sigma}{N}$$

$$RL = \frac{L_t}{T} \times 100$$

$$CI = \frac{L_s}{L_t} \times 100$$

$$AR = \frac{L_l}{L_s}$$

Table 12. Relative Length Computation (N = 76)

	1a	1b	2a	2b
ΣX	2321.60	2021.00	1830.50	1585.70
$\bar{X}$	30.95	26.95	24.41	21.14
ΣX^2	72176.62	54717.50	45102.59	33818.45
$\Sigma X^2/N$	954.37	729.57	601.37	450.91
$\bar{X}^2$	949.87	726.30	595.85	446.90
$\frac{\Sigma X^2}{N} - \bar{X}^2$	4.50	3.27	5.52	4.01
σ	2.12	1.81	2.35	2.00
S.E.	0.23	0.21	0.27	0.23

	3	4	5	X
ΣX	1461.60	764.00	426.00	1245.50
$\bar{X}$	19.23	10.05	5.61	16.39
ΣX^2	28201.60	7733.20	2435.60	20807.00
$\Sigma X^2/N$	371.07	101.75	32.05	273.77
$\bar{X}^2$	369.85	101.04	31.47	268.63
$\frac{\Sigma X^2}{N} - \bar{X}^2$	2.22	0.71	0.58	5.14
σ	1.49	0.84	0.24	2.27
S.E.	0.17	0.10	0.03	0.26

Table 13. Computation of Homologue Agreement (N = 76)

	1	2	3	4	X
ΣX	56.25	63.92	70.66	68.80	70.83
$\bar{X}$	.872	.841	.930	.905	.932
ΣX^2	58.13	54.39	66.27	62.77	67.12
$\Sigma X^2/N$	.765	.716	.872	.826	.868
$\bar{X}^2$	.760	.707	.865	.819	.868
$\frac{\Sigma X^2}{N} - \bar{X}^2$	.005	.009	.007	.007	.014
σ	.071	.095	.084	.683	1.20
S.E.	.008	.011	.010	.009	.013

Table 14. Centromere Index (N = 77)

	1a	1b	2a	2b
ΣX	2052.70	1372.60	2020.00	1897.60
$\bar{X}$	26.65	17.83	26.20	24.60
ΣX^2	56142.50	28877.30	55267.90	48903.60
$\Sigma X^2/N$	729.12	336.07	717.76	635.11
$\bar{X}^2$	710.22	317.91	686.44	605.16
$\frac{\Sigma X^2}{N} - \bar{X}^2$	18.90	18.16	31.22	29.95
σ	4.35	4.25	5.59	5.47
S.E.	0.49	0.49	0.64	0.62

	3	4	5	X
ΣX	2446.90	3554.50	3638.30	2107.10
$\bar{X}$	31.80	46.20	47.20	27.40
ΣX^2	79510.11	164561.09	172217.95	58669.73
$\Sigma X^2/N$	1032.59	2137.16	2236.59	761.94
$\bar{X}^2$	1011.24	2134.44	2231.39	750.76
$\frac{\Sigma X^2}{N} - \bar{X}^2$	21.35	2.72	5.20	11.18
σ	4.62	1.65	2.28	3.34
S.E.	0.62	0.19	0.25	0.38

Table 15. Arm Ratio (N = 77)

	1a	1b	2a	2b	3	4	5	X
ΣX	222.87	382.54	224.20	254.0	170.12	90.67	88.99	210.94
$\bar{X}$	2.89	4.97	2.91	3.30	2.21	1.18	1.15	2.74
ΣX^2	695.70	2122.96	687.49	981.75	391.48	108.00	103.90	602.37
$\Sigma X^2/N$	9.03	27.57	8.92	12.75	5.08	1.40	1.35	7.82
$\bar{X}^2$	8.35	24.70	8.47	10.89	4.88	1.39	1.15	7.51
$\frac{\Sigma X^2}{N} - \bar{X}^2$	0.68	2.87	0.45	1.86	.20	.01	.20	.31
σ	.82	1.70	.67	1.36	.447	.10	4.47	.56
S.E.	.09	.19	.08	.15	.05	.01	.05	.06

Table 16. Multipolar Abnormalities of 22 Cells[1]

	Tripolar	Tetrapolar	Heptapolar	Deletions		Bridges	
				Single	Double	Single	Double
Number	16	5	1	6	6	9	13
Per cent	72.73	22.73	4.5	27.27	27.27	40.91	59.09

[1]These 22 cells represent 4.38 per cent of the analysis in Table 1.

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